[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Biosynthesis[1] of C^{14}-Labeled Cotton Seed Oil from D-Glucose-6-C^{14}

BY F. SHAFIZADEH AND M. L. WOLFROM

RECEIVED DECEMBER 1, 1955

Direct evidence is presented to indicate that in the maturing cotton boll, D-glucose (as D-glucose-6-C^{14}) is partially converted to the seed oils, possibly through breakdown by the glycolytic process.

The origin of fats and their biosynthesis from carbohydrates in animals and microörganisms, through the formation of two carbon fragments (active acetate), has been well established by the use of carbon isotopes.[2,3] Despite this, similar investigations in the higher plants have been conspicuously lagging and consequently the present knowledge concerning the biosynthesis of oils in higher plants is still based mainly on indirect evidence such as the measurement of the respiratory quotient and the reduction in the supply of carbohydrates present during the period of rapid oil formation.

In the maturing cotton boll, according to Caskey and Gallup,[4] during the period between the twenty-first and the thirtieth day after the fertilization of the flower, rapid formation of seed oils takes place and the reducing sugar content of the boll, which consists mainly of D-glucose and D-fructose,[5,6] gradually decreases. However, as these authors themselves admit, their work does not constitute incontrovertible evidence for the formation of oil at the expense of the reducing sugars, since the decrease of these sugars in the above period is also accompanied by the rapid formation of cellulose.[5-7] In our work with D-glucose-6-C^{14}, direct evidence has been obtained which indicates that a small portion of the reducing sugars is utilized for the biosynthesis of the seed oils.

We have adapted the method of Greathouse[8] for introducing D-glucose-6-C^{14} into 21-day old maturing cotton bolls. The conversion of this sugar to cellulose with a radiochemical yield of 23.5% and the evidence for its reversible breakdown through the glycolytic process has been discussed in a previous communication.[9] The purified seed oil isolated from the treated bolls after complete maturity was also found to be radioactive. For further investigations a sample of this product was converted to glycerol and a mixture of fatty acids. The specific activities of the original oil and the mixture of the fatty acids were determined by oxidation with the Van Slyke–Folch reagent[10,11] at atmospheric pressure and radioassay of the resulting carbon dioxide as barium carbonate. The glycerol constituent was oxidized with periodate to two moles of formaldehyde, from the terminal positions and one mole of formic acid from the middle position. These fragments were separated by the method of Eisenberg,[12] converted to carbon dioxide and again radioassayed as barium carbonate. The resulting data are given in Table I. In these experiments the radiochemical yield of C^{14}-labeled cotton seed oil amounts to 3.6%.

TABLE I

THE SPECIFIC ACTIVITY OF C^{14}-LABELED COTTON SEED OIL AND ITS COMPONENTS

Radioactive moiety	Activity, μcuries/carbon atom
Cotton seed oil	9.4
Fatty acids	9.5
Glycerol	
Carbons 1 and 3	8.7
Carbon 2	2.6

It has been noted already that at least part of the radioactive D-glucose introduced into the cotton boll undergoes reversible breakdown and resynthesis through the glycolytic process, the end product of which is known to be two carbon fragments (active acetate). It seems logical to assume that this process may also be responsible for the conversion of radioactive D-glucose into the fatty acids and glycerol. The distribution of label in the latter substance also appears to be consistent with this contention.

It should be noted that the first direct evidence indicating the ability of cells from a higher plant to convert the substrates D-glucose, D-fructose and acetate into oil, has been obtained by Newcomb and Stumpf,[13] through experiments with sliced peanut cotyledon. In our experiments it is very probable that the small amount of the radioactive sugar, which is artificially introduced into the cotton boll, equilibrates with the supply of non-radioactive sugar which is present, without grossly disturbing the biochemical balance of the media and thus the incorporation of radioactive sugar into seed oil takes place under normal circumstances.

Acknowledgment.—We are pleased to acknowledge the assistance of Mr. J. R. Torres in the preparation of the radioactive cotton seed oil.

(1) This work was carried out under contract (DA-33-019-ord-1476; technical supervising agency, Ballistic Research Laboratories, Aberdeen Proving Ground, Maryland) between the Office of Ordnance Research of the U. S. Army Ordnance Corps and The Ohio State University Research Foundation (Project 591).

(2) K. Bloch, *Ann. Rev. Biochem.*, **21**, 273 (1952).

(3) H. J. Deuel, Jr., and Margaret G. Morehouse, *Advances in Carbohydrate Chem.*, **2**, 119 (1946).

(4) C. Caskey, Jr., and W. D. Gallup, *J. Agr. Research*, **42**, 671 (1931).

(5) J. Compton and E. H. Forrest, Jr., *Contribs. Boyce Thompson Inst.*, **11**, 281 (1940).

(6) A. L. Kursanov and E. I. Vyskrebentseva, *Biokhimiya*, **17**, 480 (1952); *C. A.*, **47**, 859 (1953).

(7) D. B. Anderson and T. Kerr, *Ind. Eng. Chem.*, **30**, 48 (1938).

(8) G. A. Greathouse, *Science*, **117**, 553 (1953).

(9) F. Shafizadeh and M. L. Wolfrom, THIS JOURNAL, **77**, 5182 (1955).

(10) D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940).

(11) H. E. Skipper, C. E. Bryan, L. White, Jr., and Olivia S. Huchinson, *ibid.*, **173**, 371 (1948).

(12) F. Eisenberg, Jr., THIS JOURNAL, **76**, 5152 (1954).

(13) E. H. Newcomb and P. K. Stumpf, *J. Biol. Chem.*, **200**, 233 (1953).

Experimental

Isolation of C^{14}-Labeled Cotton Seed Oil.—The seeds from ten matured cotton bolls which were treated with 33.0 μc. of D-glucose-6-C^{14} (150 mg.) as described in our previous communication,[9] were separated from the radioactive cellulose and ground in a mortar to break the hulls. The ground product (8.49 g.) was then extracted with 250 ml. of benzene in a Soxhlet extractor for three days according to the method of Koo and King.[14] The extract was evaporated and the remaining oil was washed with a 10% solution of sodium carbonate and then with water. The dark brown product was further purified[15] by adsorption on a column containing a mixture of 10 g. of Florex XXX[16] and 1 g. of decolorizing carbon, and was subsequently eluted with 200 ml. of benzene. Evaporation of the solvent furnished 2.01 g. of bright yellow cotton seed oil.

Conversion of the C^{14}-Labeled Cotton Seed Oil to Glycerol and Fatty Acids.—A portion of C^{14}-labeled cotton seed oil (1.119 g.) was saponified with 15 ml. of 0.5 *N* alcoholic potassium hydroxide according to the A. O. A. C. Official Method.[17] The reaction mixture was then treated with 1 ml. of 6 *N* sulfuric acid and evaporated. After removal of the alcohol, the residue was boiled briefly with 20 ml. of 0.2 *N* sulfuric acid and the liberated fatty acids were extracted with 40 ml. of ether. The extract was washed with three 10-ml. portions of water and evaporated to dryness; yield 1.020 g. The combined aqueous layer and the washings were neutralized and evaporated to a dry residue which was extracted with three 20-ml. portions of absolute alcohol. Removal of the alcohol by evaporation under reduced pressure furnished crude glycerol; yield 123 mg.

Conversion of Cotton Seed Oil and Fatty Acids to Barium Carbonate.—A sample of the oil (*ca.* 12 mg.) was weighed in a cup and oxidized with 0.5 g. of potassium iodate and 25 ml. of the Van Slyke–Folch reagent, by heating with a very small flame for 15 min. The resulting carbon dioxide was swept out with a current of nitrogen and was absorbed in a solution of half-saturated barium hydroxide containing 2% barium chloride. The precipitate of barium carbonate was filtered on a fritted glass crucible and thoroughly washed with water, while avoiding any unnecessary exposure to the atmosphere. It was then dried and weighed. The barium carbonate obtained in this manner from 12.5 mg. of the C^{14}-labeled cotton seed oil and 13.7 mg. of the fatty acids amounted to 157.2 and 175 mg., respectively. Under the employed conditions the amount of blank was negligible and a sample of commercial cotton seed oil containing 79.2% carbon provided a 98.3% yield of barium carbonate.

Periodate Oxidation of Glycerol.—The above isolated glycerol (123 mg.) was dissolved in 5 ml. of 0.5 *M* sodium phosphate buffer at *p*H 5.8 and oxidized with a solution of 0.65 g. of sodium metaperiodate in 10 ml. of water. The formaldehyde and formic acid produced were recovered as barium carbonate (349.6 and 174.6 mg., respectively) according to the method described by Eisenberg.[12]

Counting Methods.—The samples of barium carbonate were counted at infinite thickness, using a preflush flow counter[18] connected to a scaler,[19] and compared with a standard sample.[20] The samples were counted long enough to reduce the random counting error to ±2%.

(14) E. C. Koo and P. S. King, *Ind. Research (China)*, **5**, 137 (1936); *C. A.*, **30**, 7887 (1936).

(15) E. M. James in "Cotton Seed," A. E. Bailey, ed., Interscience Publishers, Inc., New York, N. Y., 1948, p. 706.

(16) A product of the Floridin Co., Warren, Pa.

(17) Association of Official Agricultural Chemists, "Official Methods of Analysis," 7th ed., Washington, D. C., 1950, p. 435.

(18) Radiation Counter Laboratories, Inc., Skokie, Ill.

(19) Nuclear Chicago, Chicago 10, Ill.

(20) Obtained from Tracerlab, Inc., Boston 10, Mass.

COLUMBUS 10, OHIO

[CONTRIBUTION FROM THE NORTHERN UTILIZATION RESEARCH BRANCH[1]]

Crystalline Methyl α-Isomaltoside and its Homologs Obtained by Synthetic Action of Dextransucrase

BY R. W. JONES, ALLENE JEANES, C. S. STRINGER AND H. M. TSUCHIYA

RECEIVED OCTOBER 7, 1955

A new series of reference compounds, methyl α-isomaltoside (methyl 6-*O*-α-D-glucopyranosyl-α-D-glucopyranoside) and its homologs, have been synthesized by the action of the enzyme NRRL B-512F[2] dextransucrase. The synthesis mixture was fractionated by chromatography on a carbon–Celite column. The products (D.P. 2 through 5) were isolated as pure crystalline compounds. They were identified and characterized by periodate oxidation, alkoxyl analyses, R_I values, melting points, optical rotations and X-ray analyses.

The action of the enzyme, dextransucrase from the organism *Leuconostoc mesenteroides* NRRL B-512F,[2] on sucrose has been used previously to obtain the high polymer dextran[3,4] and the disaccharides, leucrose[5] and isomaltulose.[6] In these syntheses, the glycosyl acceptors are believed to have been sucrose or D-glucose and D-fructose, respectively. A number of other carbohydrates may serve as glycosyl acceptors,[4,7] as has been demonstrated by means of paper chromatography. When maltose was used as alternate acceptor, the trisaccharide panose was prepared.[8]

This paper reports the use of methyl α-D-glucoside as alternate acceptor in the synthesis of an homologous series of sugar derivatives. Transfer of the

(1) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) *Leuconostoc mesenteroides* NRRL B-512F is a substrain of NRRL B-512. The origin of the strain B-512 and the production of dextran from it were reported by Allene Jeanes, C. A. Wilham and J. C. Miers, *J. Biol. Chem.*, **176**, 603 (1948). In 1950, the B-512F substrain supplanted B-512 for all work at the Northern Regional Research Laboratory. Since that time, the dextran from this substrain has been designated inexactly as B-512 in numerous publications. However, in this article and hereafter, the correct designation of the substrain will be used. The dextrans from B-512 and B-512F appear to be identical.

(3) H. M. Tsuchiya, H. J. Koepsell, J. Corman, G. Bryant, M. O. Bogard, V. H. Feger and R. W. Jackson, *J. Bact.*, **64**, 521 (1952).

(4) H. M. Tsuchiya, N. N. Hellman, H. J. Koepsell, J. Corman, C. S. Stringer, S. P. Rogovin, M. O. Bogard, G. Bryant, V. H. Feger, C. A. Hoffman, F. R. Senti and R. W. Jackson, THIS JOURNAL, **77**, 2412 (1955).

(5) F. H. Stodola, H. J. Koepsell and E. S. Sharpe, *ibid.*, **74**, 3203 (1952).

(6) F. H. Stodola, E. S. Sharpe and H. J. Koepsell, *Abstract Papers, Am. Chem. Soc.*, **126**, 5D (1954).

(7) H. J. Koepsell, H. M. Tsuchiya, N. N. Hellman, A. Kazenko, C. A. Hoffman, E. S. Sharpe and R. W. Jackson, *J. Biol. Chem.*, **200**, 793 (1953).

(8) Mary Killey, R. J. Dimler and J. E. Cluskey, THIS JOURNAL, **77**, 3315 (1955).